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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Lal *et al.*

Application No. 09/763,397

Filed: February 16, 2001

For: RECOMBINANT MULTIVALENT MALARIAL VACCINE AGAINST PLASMODIUM FALCIPARUM

Examiner: Vanessa L. Ford

Date: June 30, 2004

Art Unit: 1645

CERTIFICATE OF MAILING

I hereby certify that this paper and the documents referred to as being attached or enclosed herewith are being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: COMMISSIONER FOR PATENTS, P.O. BOX 1450, ALEXANDRIA, VA 22313-1450 on the date shown below.

Attorney for Applicant(s) Debra Condra

Date Mailed June 30, 2004

COMMISSIONER FOR PATENTS
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DECLARATION UNDER 37 C.F.R. § 1.131

We, Ya Ping Shi, Altaf A. Lal and Seyed E. Hasnain hereby declare as follows:

1. We are the co-inventors of the subject matter described and claimed by the patent application referenced above, *i.e.,* United States application No. 09/763,397 (hereafter the '397 application). We were employed by the Centers for Disease Control and Prevention (CDC), the assignee of the '397 application, which is located in Atlanta, Georgia, while developing the invention described and claimed in the referenced application.

2. We understand that claims pending in the present application have been rejected in view of Gilbert *et al.*, *Nature Biotechnology*, 15: 1280-1284, 1997. We understand that Gilbert *et al.*, has been cited as allegedly anticipating certain claims pending in the referenced application, or, in the alternative, as allegedly rendering the claimed embodiments obvious.

3. The publication date of Gilbert *et al.*, is November 1997. United States Provisional Application No. 60/097,703 was filed on August 21, 1998. However, we invented the subject matter covered by the claims pending in the '397 application well prior to the November 1997 date that Gilbert *et al.*, became available as a reference.

4. Accompanying this Declaration as Exhibit A are photocopies of pages from Dr. Shi's laboratory research notebook. These copes are true and accurate facsimile copies of

photocopies of the corresponding pages from Dr. Shi's laboratory notebooks. All dates stated on these pages have been redacted.

5. All entries on the notebook pages of Exhibit A were made prior to November 1997.

6. The ideas and concepts demonstrated by Exhibit A arose from work conducted for the CDC in Atlanta, GA. These ideas and concepts are embodied in the claims of the '397 application. Thus, conception and reduction to practice of the invention recited in the claims of the '397 application, as discussed in more detail below, occurred in the United States of America prior to November 1997.

7. Exhibit A consists of 21 pages of laboratory notebook pages. Exhibit B consists of one page of CDC Biotechnology Core Facility Records. The contents of these pages of Exhibits A and B, and pertinent statements made on these pages are discussed below.

A. Exhibit B is a record from the CDC Biotechnology Core Facility showing the dates of a request for oligonucleotide synthesis, and the sequences of the requested oligonucleotides. These requests were made prior to November 1997. These oligonucleotides were used as is depicted in Figure 2 of the specification to amplify the synthetic vaccine antigen gene construct using Polymerase Chain Reactions (PCRs). The oligonucleotides of Exhibit B consist of both forward and reverse complementary sequences of SEQ ID NO: 1 of the application, with overlapping sequences acting as primers for the amplification in either the forward or reverse direction.

B. Pages 1-6 of Exhibit A display the planning strategy for the PCR synthesis of the synthetic gene construct. Set forth are relevant calculations for PCR reactions and primers used to generate quantities of the synthetic gene construct. Also shown are electrophoresis gels used to visually confirm the size of PCR-generated products.

1) Page 1 shows the calculation and strategy for serial PCRs. As is set forth at the top of page one, "AA" was short hand for the PCR reaction involving oligonucleotides G0, GL, G1, and G2 of Exhibit B. "BB" was short hand for the PCR reaction involving oligonucleotides G3-G6, and "CC" was short hand for the PCR reaction involving oligonucleotides G7-G12.

2) Pages 2-5 show several experiments, ending with success as indicated by the comment "works well" on page 5. Reactions DD-II as depicted were successive rounds of PCR that joined the amplified fragments into the final synthetic gene construct.

C. Page 6 shows an electrophoresis gel of four samples at different concentrations from PCR reactions (the central four bands of the gel). Next to the gel is the comment "good!" indicating that the size of the band corresponding to the PCR product in each sample appeared to be the correct size.

D. Pages 7-10 shows that the PCR product was isolated and purified from the electrophoresis gel shown on page 6. Next, the purified product was cut with restriction endonucleases with BamH1 and Not1 (shown as steps #3 and #4 in Figure 2) for cloning into the expression vector pBluescript. The resulting sequence was SEQ ID NO: 1 of the application. Also shown on page 8 are the ligation reaction conditions for the ligation reaction, followed by restriction endonuclease reactions to evaluate the success of the ligation. The vector containing the fragment was then transformed into cells and plated onto agar plates. Positive clones were identified by their white color, indicating that the blue color-producing gene characteristic of a vector without a cloned segment had been interrupted with a cloned fragment. Page 10 sets forth the conditions for the PCR reaction to confirm that the correct gene fragment had been ligated into the vector (*i.e.,* to identify positive clones). The notations indicates that seventeen positive clones (numbers 1-4, 6, 8, 17, 21, 22, 25-27, 31, 33, 36, 39 and 40) were identified.

E. Page 11 shows an electrophoresis gel displaying samples of the PCR products.

F. Page 12 shows a single and double digesting experiment to confirm that the cloned fragment was properly oriented and was of the correct size. Clones 3, 26, and 33 were discarded by this experiment, leaving fourteen correct clones.

G. Page 13 shows the methods for the transformation of two plasmids, pBacPAK8 and pBacPAK9 with the synthetic gene construct for expression in Baculovirus. Also shown is an electrophoresis gel displaying samples of digested and undigested plasmid.

H. Page 14 shows another electrophoresis gel containing samples of DNA that were purified and digested with restriction endonucleases Not1 and BamH1, to confirm that the cloning into the Baculovirus expression vectors had been successful. The notation "orders are no problem" indicates that the clones were correctly oriented, and the statement "confirm 11, 20, 63 clones are true clones" indicates that these clones were considered to be successful. The

depicted gel shows the results of restriction endonuclease digestion showing the two bands of each clone (lanes 4, 5 and 8, respectively). Clone number 20 was identified as the clone that would be sequenced to confirm correctness at the molecular level. The sequencing indicated that clone 20 contained a single mutation. Therefore, a second clone, number 63 was sequenced. The sequencing indicated that clone 63 also contained a single mutation. In comparing the location of the mutations in clones 20 and 63, the mutations were found to be located in different segments. Thus, subsequent experiments was performed to generate a subclone that would contain the correct segments of clones 20 and 63.

I. Page 15 sets forth conditions for methylation experiments, which were run to protect restriction endonuclease sites in the vector. Following these reactions, the correct segments of clones 20 and 63 (as shown on the bottom of pg. 15) were excised and ligated into the Baculovirus expression plasmid pBacPAK8.

J. Page 16 shows a basic diagram of the recombinant vaccine antigen gene as cloned into the Baculovirus expression vectors. As shown, the construct contains portions of both clones 20 and 63.

K. Page 17 shows a gel wherein the products of the second ligation reaction of the correct segments of constructs 20 and 63 into the pBacPAK8 expression vector were run to confirm the size of the construct.

L. Page 18 shows the success of a ligation experiment, as confirmed by visualization of the bands on the electrophoresis gel. The statement that "clones 21, 31, 33, 35 are positive" indicates that the ligation reaction was successful.

M. Page 19 shows an electrophoresis gel displaying the results of BamH1 restriction endonuclease digestion, to confirm the successful clones.

N. Page 20 shows an electrophoresis gel displaying the results of BamH1 and Not1 restriction endonuclease digestion. The notation "save clone 31A and 31B" indicates that these clones were successful. These results were subsequently confirmed by sequencing clones 31A and 31B.

O. Page 21 contains the notation "miniprep for sending product to Hassian (sic)." This refers to co-inventor Dr. Seyed Hasnain, who tested the expression of the synthetic gene construct in the Baculovirus expression system.

8. All statements made herein and of our own knowledge are true and all statements made on information are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

06/14/2004 — Ya Ping Shi

Date — Name Ya Ping Shi

Date — Name Altaf A. Lal

Date — Name Seyed E. Hasnain

8. All statements made herein and of our own knowledge are true and all statements made on information are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date	Name Ya Ping Shi
Date June 14th, 2004	Name Asfaf A. Lal
Date	Name Seyed E. Hasnain

8. All statements made herein and of our own knowledge are true and all statements made on information are believed to be true. Furthermore, these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements made may jeopardize the validity of the application or any patent issuing thereon.

Date	Name Ya Ping Shi
Date	Name Altaf A. Lal
June 21, 2004	Seyed Hasnain
Date	Name Seyed E. Hasnain

EXHIBIT A

First PCR

	Tm
AA: G2 - G3	52.5
BB. G3 - G6	58.5
CC. G7 - G12	61.5

94°C 5min
94°C 45"
45°C 1min
72°C 1.5min. 8 cycle (p139)

16ul dNTP
1 ul Buffer
0.5ul Taq
26.5 ul.

5 strong/each. 73.5

AA: 2×4 = 8ul 65.5

BB 2×4 = 8ul 65.5

CC. 2×6 = 12ul 61.5

---

second PCR. 53.5

16ul dNTP
10ul buffer
0.5ul Enzyme
5ul Olig 1
5ul Olig 2
46.5

94°C 5min.
94°C 45" → 25 cycle
45°C 1min
72°C 1.5min (P141)

AA
- DD1 1ul 52.5+5+5
- DD2 2.5ul 51+5+7 → G0
- DD3 5ul 48.5+5+5 G2
- DD4 10ul 43.5+5+5

BB
- EE1 1ul 52.5+5
- EE2 2.5ul 51+5 → G3
- EE3 5ul 48.5+5 → G6
- EE4 10ul 43.5+5

C
- FF1 1ul 52.5+5
- FF2 2.5ul 51+5 → G7
- FF3 5ul 48.5+5 G12
- FF4 10ul 43.5+5

Redo CC_0: G7 - G12 = 12ul.

dNTP	16ul c. Taq
10× Buffer	10ul.
H_2O	61.5ul
	100ul

94°C 5min
94°C 45" 〉8 cycle.
40°C 1min
72°C 2min

CC_1' G7 - G8 (only do second PCR) = 4ul + 69.5ul H_2O.

CC_2' G9 - G12 2×4 = 8ul + H_2O 65.5 _

Do SOE G6 - G76

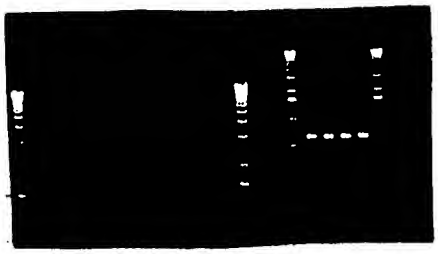
	DD1 + EE1	H_2O	63.5	
GG1	1ul + 1ul = 2ul	61.5		16ul dNTP.
GG2	2.5ul + 2.1ul = 5ul	58.5		10ul Buffer.
GG3	5ul + 5ul = 10ul	53.5		5ul G0
GG4	10ul + 10ul = 20ul	43.4		5ul G76
				0.5 Taq
				36.5

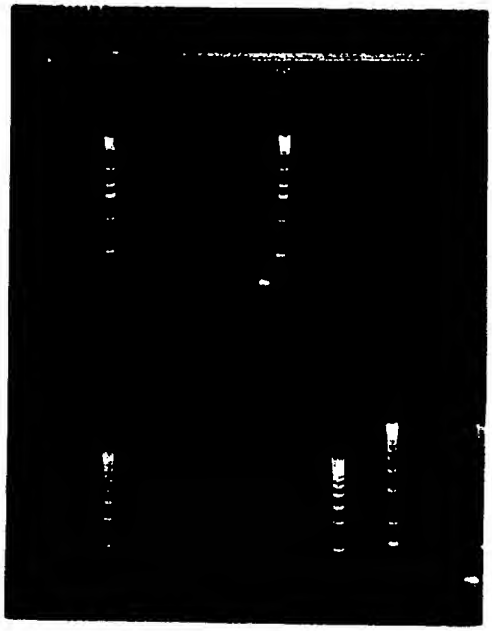
program 141

		63.5		
FF1	primers	1ul	H_2O 62.5	16ul dNTP
FF2 (CC1')	G7	2.5ul	61	10ul Buffer
FF3	G12 10ul	5ul	58.5	oligos 10ul.
FF4		10ul	53.5	Taq 0.5
FF5		1ul		36.5
FF6 (CC2')	primers	2.5ul		94°C 5min
FF7	G9	5ul.		16°C 45"
FF8	G12 10ul	1ul		40°C 1min
				72°C 2min

x cycles #41

Result: GG1-4





F1-3 did not work probably because oligo ?

Prepare new temp oligo G4 - G12. also AL-1065.

Redo: $CC'_2 \rightarrow CC''_2$ and CC''_3.

CC''_2 G9 G10 G11 G12 × 2 = 8ul · 65.5 → 3.5

GG''_3 G9 G10 G11 AL1064 ×2 = 8ul · 65.5

× works well

16 ul dNTP.
10 ul Buffer.
0.5 Taq

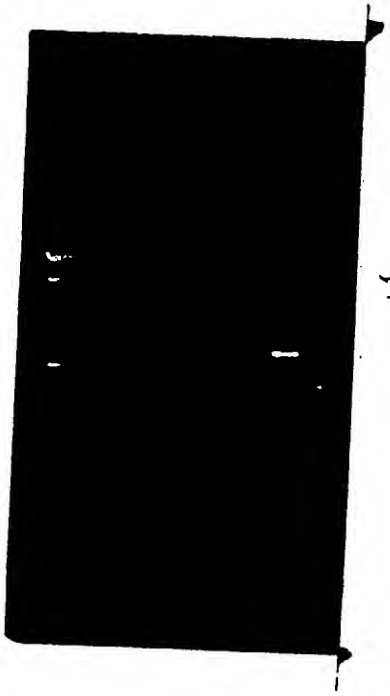
Same to before.

Second PCR.

FF''_1	CC''_2	primers	1ul	H_2O 62.5	16ul dNTP
FF''_2		G9	2.5ul	61	10ul buffer
FF''_3		G12	5ul	58.5	61/80 10ul
FF''_4			10ul	53.5	Taq 0.5ul.
FF''_5	CC''_3	G9			
FF''_6		AL1064			
FF''_7		works well			
FF''_8					

Same to before.
11/4/

II 4



II

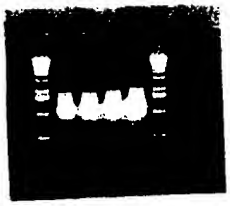
SOE for G17 - G1 + AL-1065.

	CC1	FF1	H2O	65.5	16ul dNTP
HH1	1ul	+ 1ul	61.5		10ul buffer
HH2	2.5ul	+ 2.5ul	58.5		5ul G7
HH3	5ul	+ 5ul.	53.5		5ul AL-1065
HH4	10ul	+ 10ul	43.5		0.5 Taq
					365

program #41

	GG1	+ HH1,	H2O	63.5	16ul dNT
II1	1ul	+ 1ul	61.5		10ul buffer
II2	2.5ul	+ 2.5ul	58.5		5ul AL1064
II3	5ul	+ 5ul	53.5		5ul AL1065
II4	10ul	+ 10ul	43.5		0.5 Taq
					365

pro#41



good!

Further cleaning and cloning,

Sequencing.

A: Run gel and cut [redacted] and clean.

① gene clean (from product of PCR)

② gel clean through column (according instruction of manufacturer) (50ul of PCR product tube one is pellet (store in -20°C)

another [redacted] has 20ul water. From this, 10ul was take for digestion.

B. digestion:

Not 1 : 26ul water.
3ul Buffer
1ul NotI

1h 37°C

(II, gene clean
II, column clean)

pellet.

↓

BamHI 26ul H2O
3ul buffer
1ul BamHI

1h 37°C

Ligation.

			Control I	Control II
Water	13ul		"	15ul
Vector	1ul	(BamHI and NotI digest)	"	
5x lig buffer	4ul.		"	
T4 ligase	2ul		"	x

over night (4°C

Not I digestion:

Vector:

1ul	Vector (uncut 3.2ug/ul)
3ul	10x buffer.
3ul	BSA.
4ul	Not I
10ul	H2O

30ul 37°C 1.5h.

target

II 2ul control (MSp-1)

23ul	H2O.
3ul	BSA.
3ul	10x Buffer
1ul	Enzyme

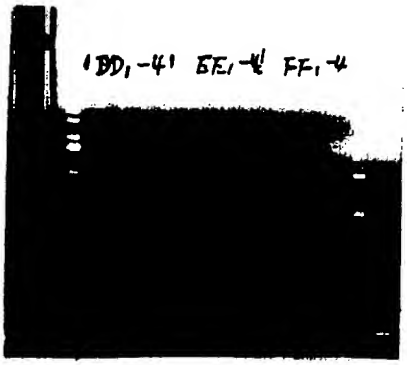
30ul 37°C 1.5h.

BamHI digestion

Vector	BamHI	4ul
	Buffer	3ul
	water	23
		30ul 37°C 1.5h.

	[illegible]	2ul
	Buffer	3ul.
	water	25ul
		30ul 37°C 1.5 hr

Result



FF4-4 did not work because first PCR (CC) annealing temp was too high

Need redo CC (first PCR), then FF1-FF4

[redacted]

Ligation as before
Transformation as before

result. not so much white clones. probably vector was not properly digested.
Change further purify vector.

pick up 40 clone grow overnight.
Cell PCR: as regular. 10ul cell 94°c 5min.

Oligo	AL1064	2.5ul
	AG1065	2.5ul
	Buffer	5ul
	dNTP	8ul
	Taq	0.5
	H2O	21.75
		40ul

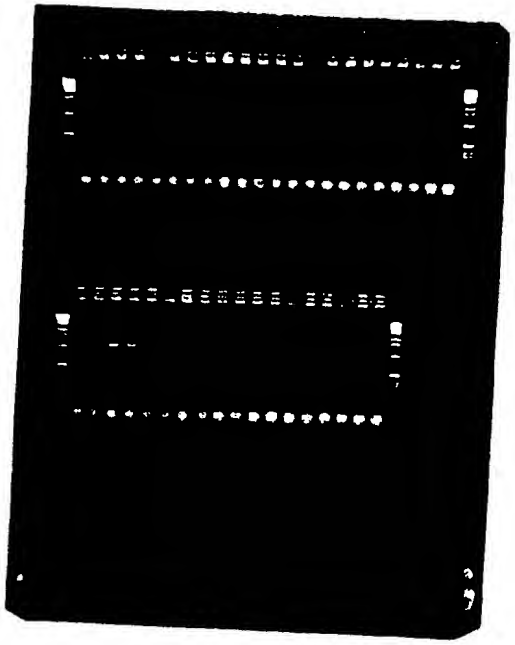
15 cycle 94.°c 45" 50°c 45" 72°c 2m

positive Clone:

1, 2, 3, 4, 6, 8, 17, 21, 22,
25, 26, 27, 31, 33, 36, 39, 40,

Grow back

[redacted]



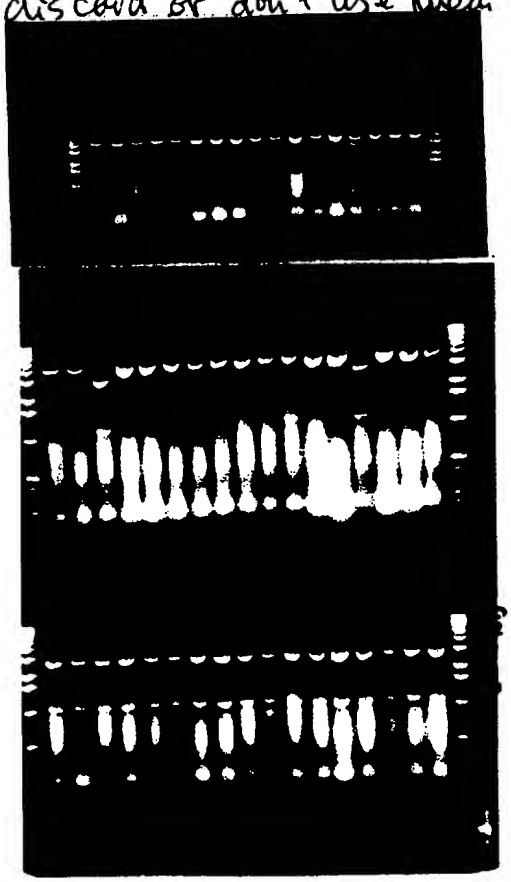
Save cell

Save as SGV/R# Synthetic gene Vaccine
patient consign

digest all positive (17) clones (based on pcR)

Single digestion : BamHI or NotI
double digestion BamHI and NotI.

Result: Clone 3. 26. 33 are not pure clones.
discard or don't use them



2.6

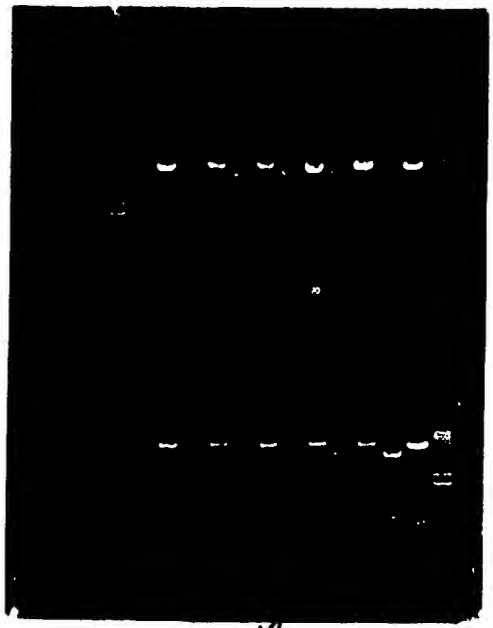
[redacted]

Plasmid pBacPAK8 and pBacPAK9 (from Sayo
2ng/100ul 2ng/100ul

Transformation:
10ul plasmide (200ng)
100ul XL-blue cell
procedure as regular.

plating: overnight
growth well

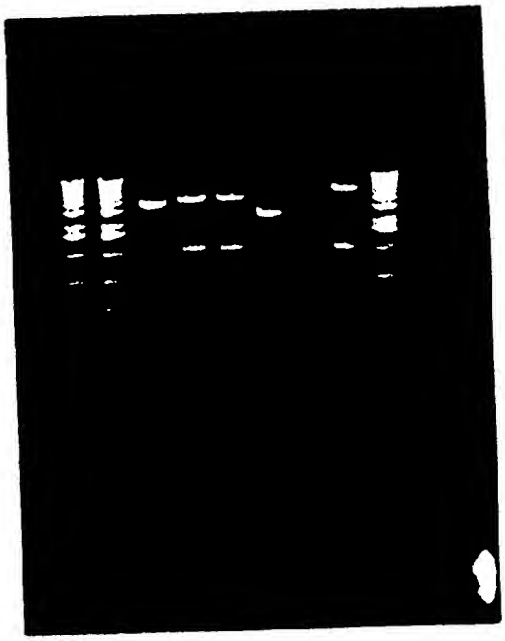
Miniprep of pBacPAK8 and pBacPAK9 -
run undigested and digested plasmid



100ng/ul ×19 =
1.9ul.

[redacted]

This result confirm that. orders are no problem. also confirm (11) (20) (63) clones are true clones.



I will sequence clone 20.

Methylation :

Clone 63 Vector correct.
Clone 20 Nest target correct.

Clone 63 methylation.

Fication:	3 ul	TaqI methylase
	3 ul	NEB 4 Buffer.
	0.3 ul	BSA.
	22.2 ul	1+20
	1.5 ul	Mix SAM

1 h 65°C

mix : 50ul NEB4 Buffer + 450ul 1+20 + 1.25ul SAM

0.6 ul Nacl (5M)
60 ul Ethanol (100%)

HInd II cut clone 63 (two piece vector bj
clone 20 (more mess vector small

Run gel Standard 20 63 Standard
(more small (two bj)

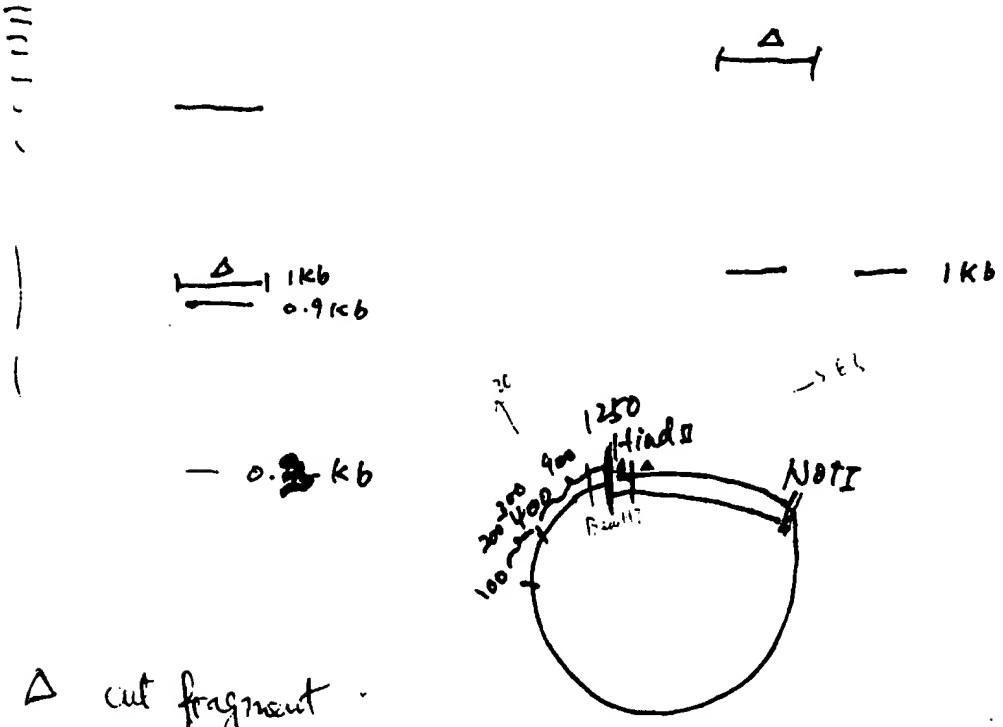
Digestion condition:

3 ul	buffer
6 ul	HindI
21 ul	H2O

1.5h 37°C

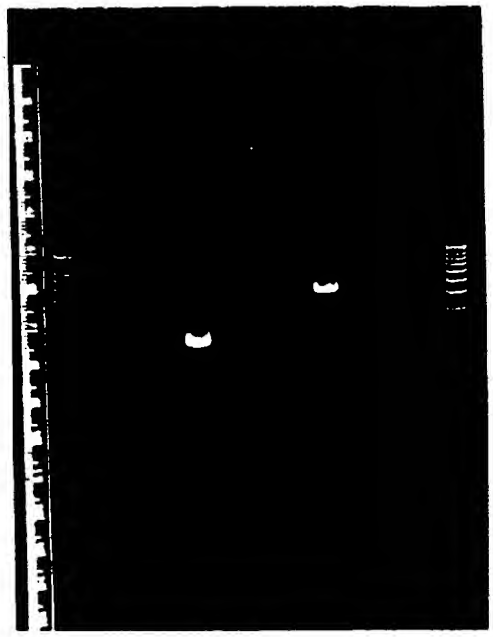
Result:

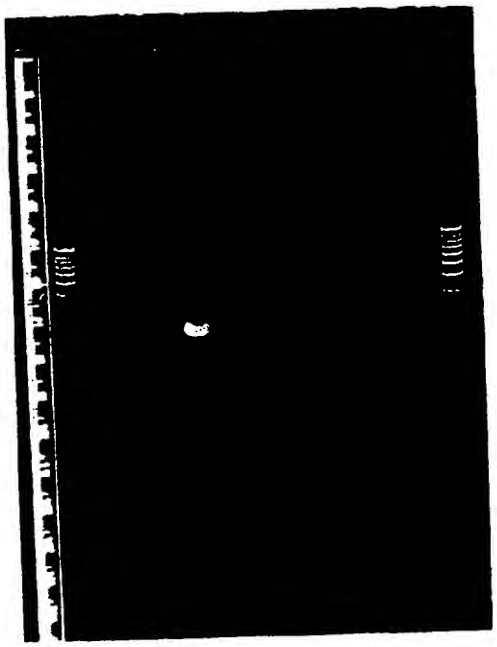
Clone 20 | Clone 63



△ cut fragment.

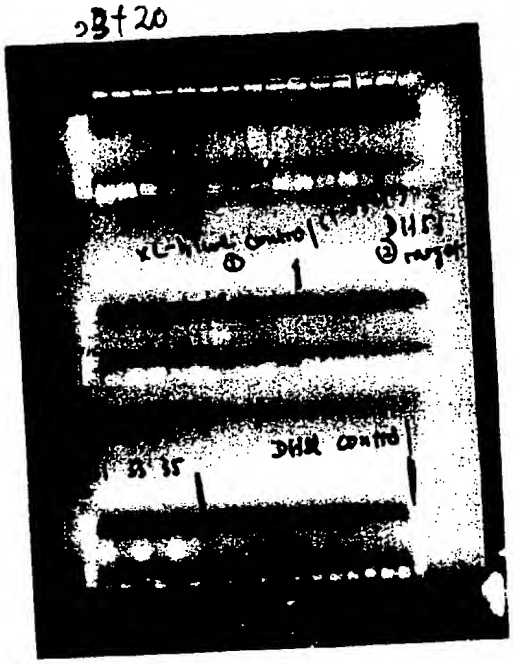
ligation: as routine





clone 63 + 20 ligation see before

clone PCR primer: AL1097
AL1064



clones 21, 31, 33, 35 are positive.

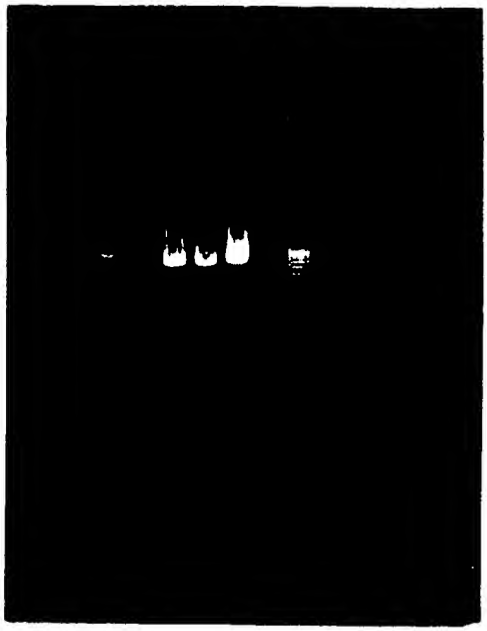
save as name:

~~38cells/cl=DT21/63+20~~

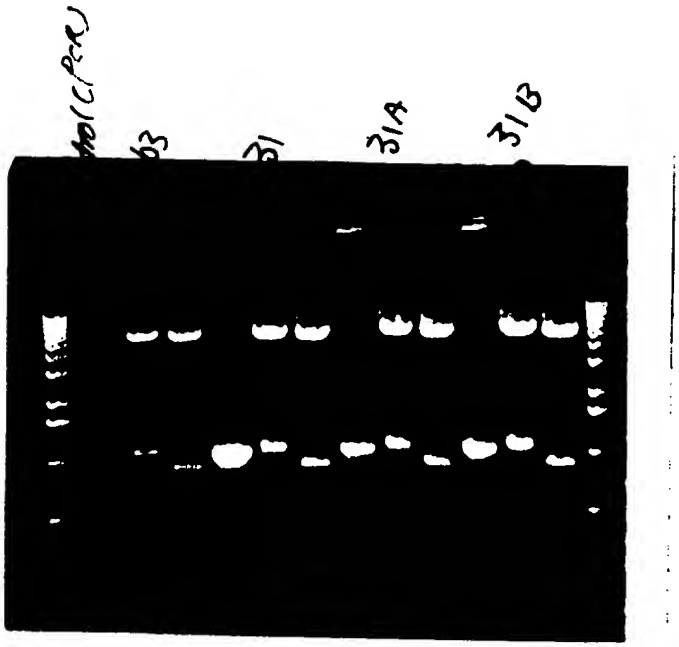
name

Pac8/63+20/number

BamHI digestion: 63+20 (21, 31, 33, 35) bp



Partial restriction methylation



Save clone 31A and 31B

named as pac8/63+20/31A and 31B

[illegible] for sending product to Hassian.

clone pneg/63+re/31A

EcoRI and NotI digestion

Send to Hassian